THE EFFECTS OF BLOOD PROTEIN DEPLETION ON THE GROWTH OF THE OOCYTES IN THE CECROPIA MOTH

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During the final two weeks in the metamorphosis of the Cecropia moth the oocytes enter a period of yolk formation, in the course of which their volume increases 400-fold. The growth of the oocyte during this period is not dependent exclusively on the synthetic activities of the ovary, but also entails the transfer of proteins from the blood to the yolk (Telfer, 1960). An analysis of the mechanism of protein transfer and its role in the formation of yolk has been undertaken. We describe here some effects of depleting the blood of two prospective yolk proteins on the growth of the oocyte and on the composition of its yolk. Advantage was taken of the decrease which normally occurs in the concentration of the two proteins during the period of yolk formation.

The growth rate of the oocyte is interpreted here as an index to its rate of yolk formation. The Cecropia oocyte belongs to that class of eggs in which the yolk inclusions constitute the primary mass of the cytoplasm: centrifugation at 20,000 $\times$ g yields a stratum of protein yolk spheres which occupies more than 90% of the volume of the oocyte. In such a case, gross changes in the rate of yolk formation should be conspicuously reflected in the growth rate of the oocyte.

Determinations of the growth rate of an oocyte, as with other internal structures of an organism, are handicapped by difficulties in measurements of the time axis. In the present case, it would be desirable to measure the individual oocyte at successive times in its period of yolk formation. However, the oocyte must be dissected from the moth in order to be measured and its capacity for further development is thereby destroyed. A second approach would be to measure the size of the oocytes in a large number of ovaries which varied only in the chronological stage of their development, and this procedure proved feasible for the Cecropia oocyte. The correlation between ovarian development and the stage of the moth's metamorphosis was found to be sufficiently precise so that we were encouraged to estimate the time of yolk formation from the directly observable time of metamorphosis.

Methods

1. *Determination of the time of development*

The sequence of developmental changes used in determining the time of the moth's metamorphosis is described in Table I. The earliest developmental stage recognized with precision was the darkening of the tarsal claws which can be seen through the pupal cuticle with the aid of a dissecting microscope. In Schneiderman and Williams' (1954) timetable for the development of Cecropia males, tarsal claw

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darkening is listed as occurring 13 days after the initiation of the pupa's development into the moth. We arbitrarily designated this stage as day 13 for the female also, and subsequent stages of development were timed accordingly. Our timetable is based on the rate of development at 25° C., the temperature at which all animals were maintained.

2. Dissection of the ovaries and determination of oocyte volume

The ovaries were dissected from 70 females in the last half of their transformation from pupae into moths. The weights of the animals dissected were within the range of 4.5 to 5.5 grams, and it should be emphasized that the timing and magnitude of the changes described are probably valid only for animals within this size range. The final stages of the dissection and measurements of the size of the oocytes were completed with the ovaries immersed in 0.15 *M* NaCl. While the measurements were generally completed within an hour after the dissection had

TABLE I

Characters used in determining the time of development of the female pupa into a moth

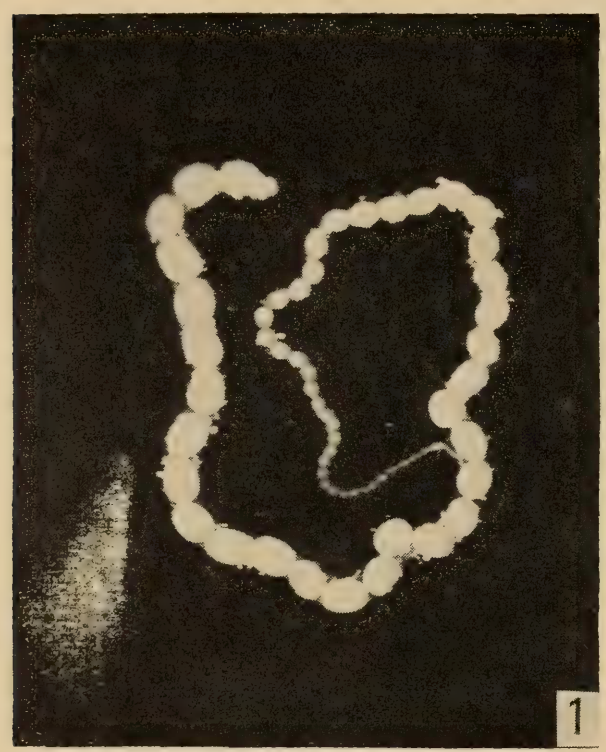
Days of development at 25° C.	Diagnostic characters
13	Tarsal claws darken
13–14	Antennal rami darken
15–16	Spines on genitalia turn from white to reddish brown
16–17	Developing ovarian tracheae become lined by a cuticular thread demonstrable by teasing the tracheae apart
18	Ovarian tracheae become air-filled and thus appear silvery
18–19	Initiation of fore-wing pigmentation: lateral spots appear
19	Advanced wing pigmentation; lateral abdominal pigmentation visible
21	Cuticle softened over facial region
22	Cuticle crisp
23	Eclosion

been begun, neither swelling nor shrinking of the oocytes was visible in ovaries which had remained for as long as 4 hours in the saline solution.

The paired ovaries contain a total of 8 tubular ovarioles, one of which was selected for study from each animal. Since there was little obvious variation among the ovarioles with regard to the number or size of the oocytes they contained, the ovariole to be studied was selected at random. The only exceptions to this procedure were the omission of occasional ovarioles which obviously differed from their seven partners.

A single Cecropia ovariole generally contains from 30 to 50 oocytes in varying stages of development (Fig. 1). Their volumes were estimated, beginning with the most mature oocyte, which is located at the posterior end of the ovariole, and ending with the smallest oocyte which contained visible yolk. The presence of yolk was recognized through a dissecting microscope by the fact that it renders the cytoplasm opaque and colors it yellow. The termination of growth coincides with the secretion of the chorion. This event was detected by two criteria: since the chorion is opaque and colorless, it causes the oocyte to appear progressively paler as it is laid down over the yellow yolk; in addition, the chorion forms a rigid crust on the surface of the oocyte which thus loses its elastic response to probing.

The volume of an oocyte was calculated from measurements of its linear dimensions. The shape of a growing oocyte approximates an ellipsoid with two equal axes. The disparate axis is the longitudinal one, or that which coincides with the axis of the tubular ovariole (Figs. 1 and 2). Although the nurse cells accompanying each oocyte tend to flatten its anterior end (Fig. 2) and thus cause some deviation from the shape of an ellipsoid, this effect is progressively reduced as the oocyte enlarges and is undetectable during the last three-quarters of the volume increase. Therefore, the volume of a growing oocyte could be estimated by measuring its longitudinal and transverse diameters with a filar micrometer and by solving the appropriate equation for the volume of an ellipsoid.



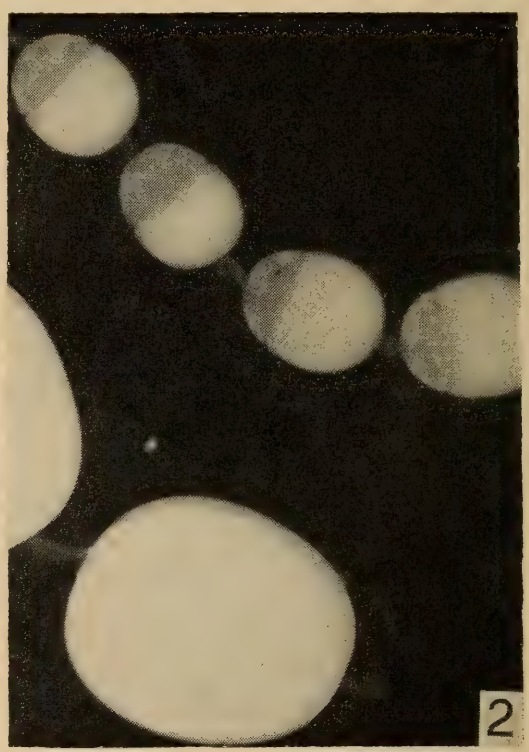


FIGURE 1. An ovariole dissected from a female Cecropia on the seventeenth day of its transformation from a pupa into a moth. The individual oocytes lie in sequence with the largest at the posterior end of the ovariole. Small fragments of fat body adhere to the outer wall of the ovariole.

FIGURE 2. Several oocytes which were removed from the ovariole shown in Figure 1. The five nurse cells form a transparent cap at the anterior end of the smaller oocytes. At the lower left is an oocyte which is nearing maturity. Magnification, 20 ×.

The most convenient index to the size of the mature oocyte was its weight, as determined with a semi-micro balance. Weighing was possible in this case because the rigid and comparatively impervious chorion allows the egg to be blotted and weighed without the loss of significant quantities of water from the oocyte itself.

As in the case of the growing oocyte, the volume of a mature oocyte could also be calculated from measurements of its diameters. The shape of the mature oocyte differs from that of the growing oocyte in that its two transverse diameters

are unequal. By means which are not apparent, one of the transverse diameters increases at the expense of the other during or shortly before the process of chorion formation. The longitudinal diameter appears to be unaffected by this process. Thus, the volume of a mature oocyte was calculated with the formula for the volume of an ellipsoid with three differing diameters.

3. *Determinations of blood protein concentrations*

The concentrations of two antigenically defined proteins in the blood and in oocyte extracts were determined with the antiserum-agar technique of Oudin. The two proteins have been described previously (Telfer and Williams, 1953). They included antigen 3, one of several carotenoid proteins in the blood, and antigen 7, a sex-limited blood protein which occurs primarily in female pupae and moths. The validity of the Oudin technique for the determination of the relative concentrations of these proteins has been discussed elsewhere, and in the case of the female protein, has been confirmed by independent methods (Telfer, 1954).

4. *Ovarian transplantations to males*

Comparisons of the effects of male and female blood on the size of the oocytes produced were made by transplanting ovaries to males which were then transfused with samples of either male or female blood. These experiments were performed on pupae of the Polyphemus moth. The method of CO_2 anaesthesia and other surgical procedures used are described in detail by Williams (1959). The tip of the abdomen was cut off from each male pupa and up to 0.5 of blood was removed through the resulting orifice by compressing the anterior end of the pupa. An ovary with a small amount of associated fat-body was dissected from a female pupa and inserted through the abdominal orifice in the male. The haemocoel of the male was then filled from a pipette with blood which had been obtained either from female pupae or from other male pupae. Finally, a piece of plastic coverslip was sealed over the opening with paraffin. The implanted ovary was dissected from the host at the conclusion of metamorphosis.

Results

1. *The volumes of the oocytes in individual ovarioles*

The stage of development of an ovariole and its oocytes can be described by plotting the volume of the successive oocytes as a function of their position in the ovariole. Three examples of the relationship in the developing Cecropia ovariole are shown in Figure 3. In all three cases oocyte volume decreased progressively along the sequence of oocytes. Exceptional in this respect were the volumes of the first 12 oocytes in the moth which had been developing for 20 days. The fact that these oocytes were nearly equal in volume can be attributed to their having completed growth, as was indicated by their all having undergone some degree of chorion formation. The three examples typify the gradient in developmental stage seen in an ovariole during the period of yolk formation. Profiles such as these for animals in varying stages of metamorphosis contain the information required for constructing growth curves for the oocyte in any particular numerical position.

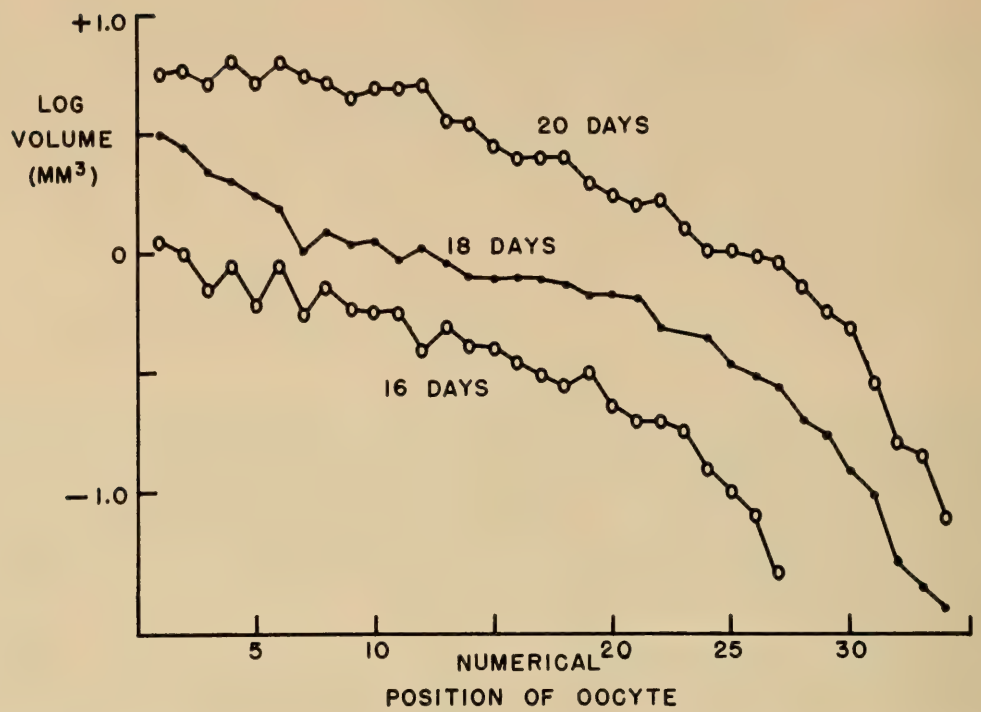


FIGURE 3. The logarithm of the volume of successive oocytes in three ovarioles. The stage of development of the female from which the ovariole was dissected, is indicated next to each curve and is expressed in days at 25° C. since the initiation of the pupal-adult molt.

2. *Oocyte growth curves*

The volume of the first, or most mature oocyte in each ovariole studied was plotted as a function of the morphologically determined age of the insect from which it had been dissected. The resulting data indicated that the logarithm of oocyte volume increases approximately linearly with time (Fig. 4) and then ceases abruptly at the time of chorion formation. Thus the growth of the first oocyte is described here as follows:

$$V = ae^{2.3bt}, \text{ or } \text{Log } V = \text{Log } a + bt, \qquad (1)$$

in which V is the volume of the oocyte in mm.^{3}, t is the stage of metamorphosis expressed in days at 25° C., and a and b are constants. Analysis of the data by the methods described in Table II yielded the information that, during the one-week period for which data were obtained, the percentage relative growth rate of the first oocyte was $65 \pm 6\%$ per day while its percentage increment in volume was 91% per day. Thus, the first oocyte nearly doubles its volume on each of at least 7 successive days of yolk formation.

3. *Comparisons of successive oocytes in the ovariole*

Growth curves constructed for the tenth, twentieth and thirtieth oocytes were similar in form to that of the first oocyte. However, the growth constants differed in such a manner as to suggest that yolk formation is less vigorous in the more anteriorly located oocytes.

Each oocyte completes its growth at a later time than its more posterior neighbor. From the data on the time at which yolk formation is completed

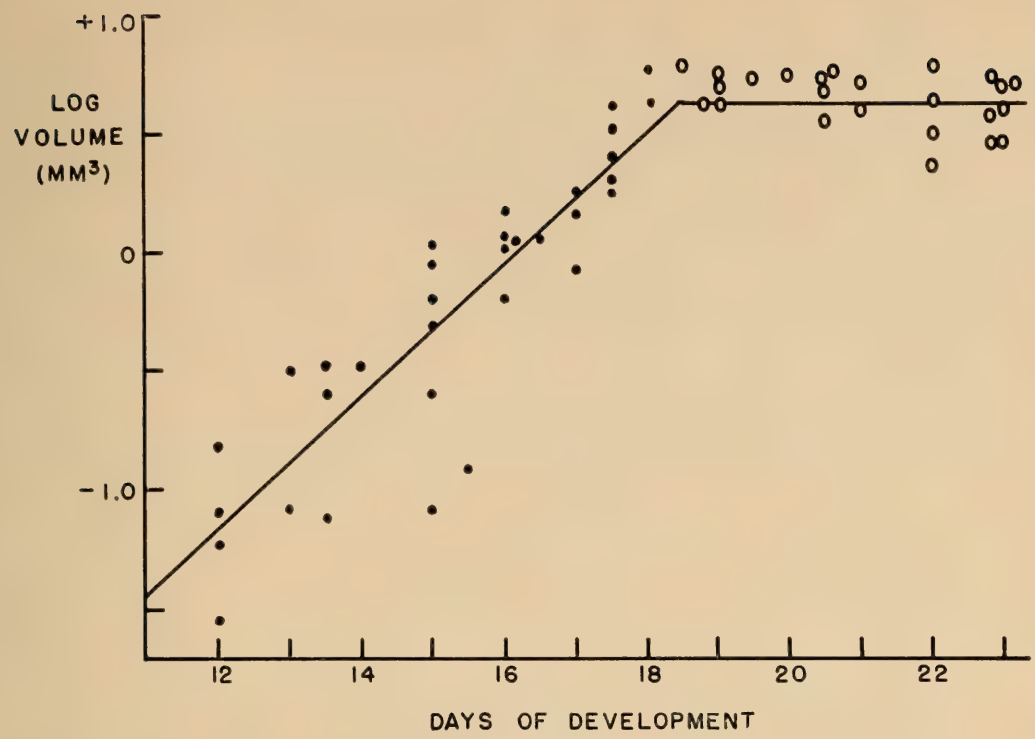


FIGURE 4. Growth curve of the first or most posterior oocyte in the ovariole of Cecropia. The logarithm of the volume of the oocyte is plotted against the time of development of the moth. Chorionated oocytes, ——○——; oocytes without chorions, ——●——.

(Table II), it can be calculated that in the ovariole as a whole, each oocyte completes yolk formation an average of 5.2 hours after its predecessor. However, there is a tendency for the first oocytes produced to follow each other in more rapid succession than the later oocytes. The average lag between successive oocytes was 4.2 hours for the first 20 oocytes, and was estimated to be 7.2 hours for the twentieth to thirtieth oocytes.

Also varying with numerical order in the ovariole is the volume of the oocyte at maturity. The volume of the first oocyte was 130% of the volume of the tenth

TABLE II

Growth characteristics of oocytes during yolk formation

Growth characteristic and its method of calculation		Numerical position in ovariole			
		1	10	20	30
Mean volume (± s.e.) of oocytes possessing chorions	($mm.^3$)	4.75 ±0.25	3.64 ±0.13	3.35 ±0.46	3.0
b (equation 1); calculated by the method of least squares for the non-chorionated oocytes		0.28	0.23	0.21	0.18
Percentage daily increment in volume [(Antilog b) −1] 100%		91%	70%	62%	51%
Percentage relative growth rate (2.3 b) 100%	(% per day)	65%	53%	48%	41%
Time when growth terminates: the value of t (equation 1) when V is the mean volume of the oocytes possessing chorions	(days)	18.6	20.1	21.9	24.9

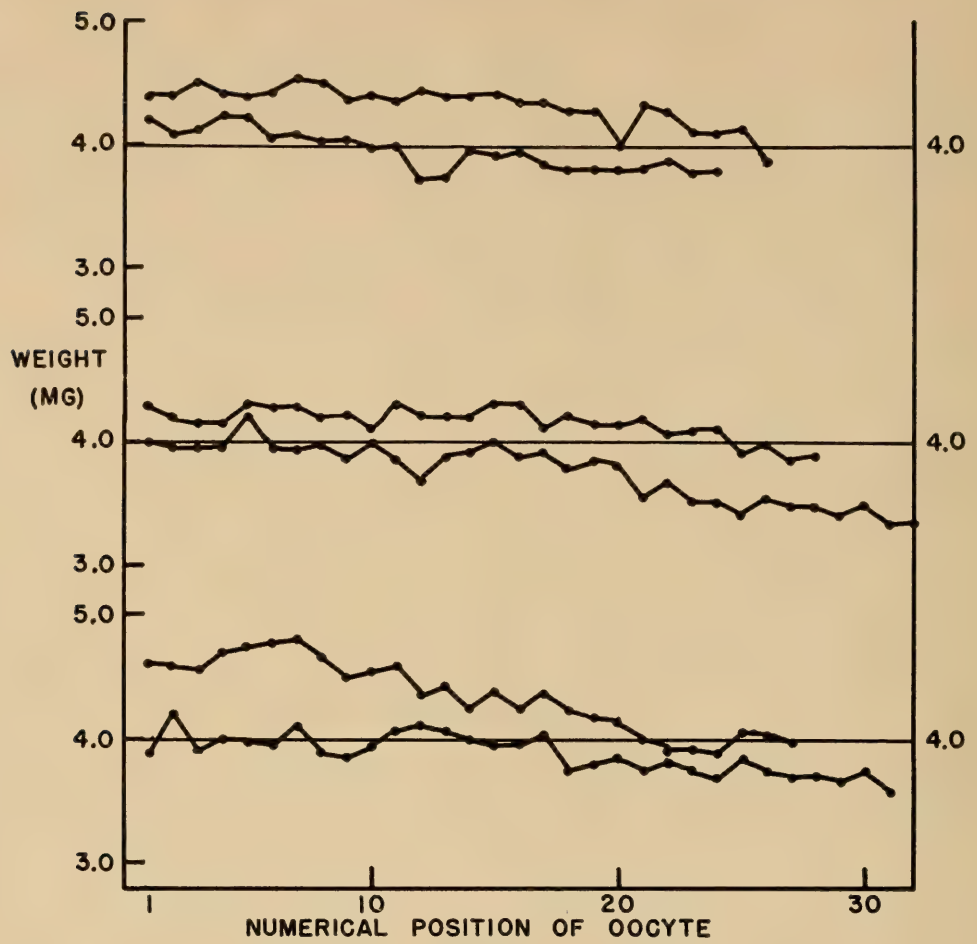


FIGURE 5. The weights of mature oocytes dissected from moths which had completed metamorphosis. The weights of successive oocytes from six different moths are plotted as a function of their numerical position in the ovariole. The reference lines are arbitrarily placed at 4.0 mg.

oocyte and 142% of that of the twentieth oocyte. The number of chorionated thirtieth oocytes was too small to permit the comparison to be extended to this case. The relationship between size at maturity and position in the ovariole was seen in a more exact manner by weighing the successive oocytes in mature ovarioles dissected from fully developed moths. In all six cases illustrated (Fig. 5), the later oocytes tended to be smaller than the first oocytes produced.

TABLE III

Comparison of the b-value for the first oocyte with those for numbers 10, 20 and 30

Numerical position of oocyte	$b \pm$ s.e.	N	Significance of difference from first oocyte (p)
1	0.28±0.025	33	—
10	0.23±0.025	41	0.11
20	0.21±0.023	43	0.04
30	0.18±0.026	28	0.008

Chi square test of the significance of the over-all decline; $p = 0.003$.

The value of b, and consequently the rate of growth, also decreases with increasing numerical position (Table II). The difference in b between the first and the thirtieth oocytes represents a decline in the percentage relative growth rate from 65% to 41% per day. Statistical comparisons indicated that the decline observed in b was significant (Table III).

Thus, all of the growth characteristics considered here change in such a manner as to suggest that yolk formation occurs with reduced vigor in the later oocytes produced in an ovariole. The question then arises as to whether this is a direct consequence of a depletion in the supply of prospective yolk proteins available in the blood.

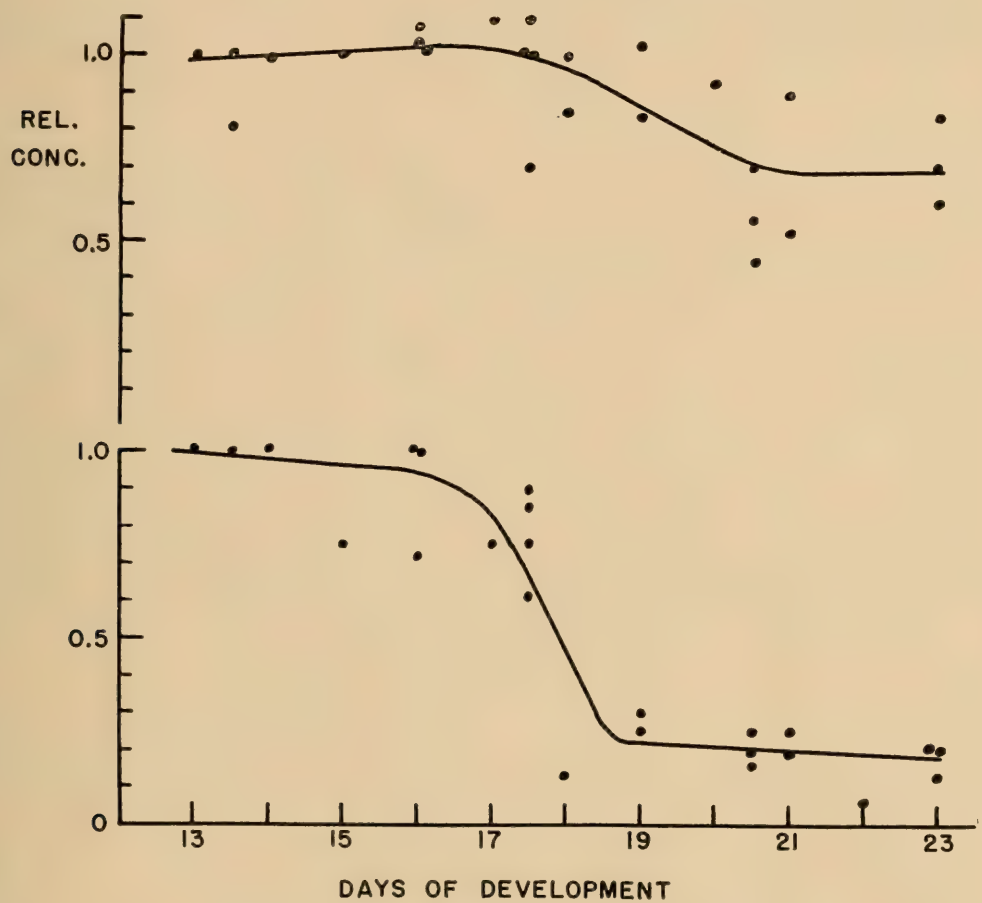


FIGURE 6. Changes in the relative concentrations of two proteins in the blood during the last ten days of the pupal-adult transformation. Upper curve is for antigen 3, a carotenoid protein. Lower curve is for antigen 7, the female protein. A relative concentration of 1.0 is arbitrarily set as the maximum concentration of each protein in the blood.

4. *Changes in the concentrations of two blood proteins during yolk formation*

Most, and possibly all, of the moth's blood proteins are deposited in the yolk to some extent, and of those studied quantitatively, the female protein is the most avidly accumulated (Telfer, 1960). It would seem reasonable, therefore, that depletion of the available blood proteins, and of the female protein in particular, could affect the growth characteristics of the oocytes. That the concentration of the female protein in the blood decreases during the last half of the transformation

of the pupa into a moth has already been demonstrated (Telfer, 1954). A more precise determination of the timing of the decrease is described here, as well as a comparison with the less readily accumulated carotenoid protein.

The results indicate (Fig. 6) that the concentration of female protein decreases approximately 80% during the period of yolk formation. The concentration of the carotenoid protein in the blood also drops during yolk formation, but in this case the decrease is not nearly as great. The difference between the two proteins in this regard is consistent with the greater extent to which the oocyte accumulates the female protein.

The timing of the decrease in female protein with relation to the growth of the four oocytes studied is summarized in Figure 7. While the first oocyte undergoes the final three-quarters of its growth (left hand cross-hatched area in Figure 7)

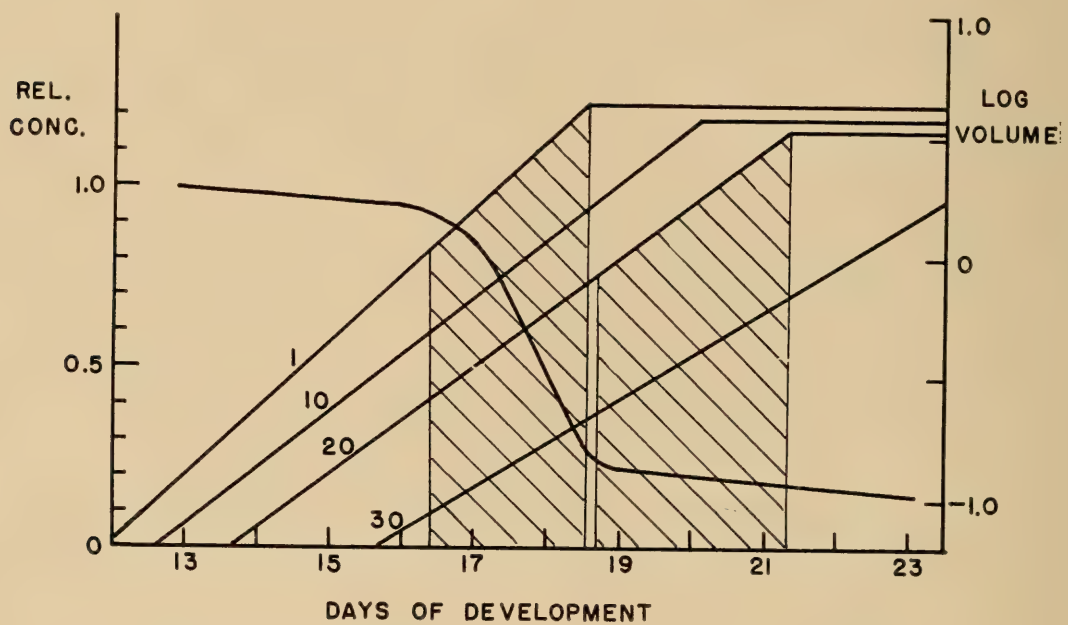


FIGURE 7. Changes in concentration of the female protein (left-hand scale) superimposed on the growth curves of the first, tenth, twentieth and thirtieth oocytes (right-hand scale). Cross-hatched areas indicate the time during which the first and twentieth oocytes undergo the final three-fourths of their volume increase.

the average female protein concentration approximates one half or more of its maximum level in the blood. The twentieth oocyte, on the other hand, undergoes the final three-quarters of its volume increase (right hand cross-hatched area) when the blood contains only one-fifth of its maximum concentration of female protein. It thus appears that the first oocytes are produced in an environment in which the concentration of female protein is greater than that of the later oocytes, and we are left with the possibility that this accounts for the reduced vigor with which the later oocytes produce yolk.

5. *The effects of a protein's concentration in the blood on its rate of accumulation by the oocyte*

The most likely mechanism by which a reduction in blood protein concentration could retard the process of yolk formation would be through a decrease in the rate of protein accumulation by the oocyte. We therefore sought to determine whether

the rates of female and carotenoid protein accumulation are affected by the decrease which normally occurs in the concentrations of these proteins in the blood. The information required here, in addition to the growth rates of the successive oocytes, was the amount of each protein accumulated per mg. of oocyte.

Successive pairs of chorionated oocytes dissected from fully matured ovarioles were weighed and then extracted with 0.2 ml. of 0.15 *M* NaCl buffered at neutrality. Either the oocytes or the extracts were frozen and thawed once, a

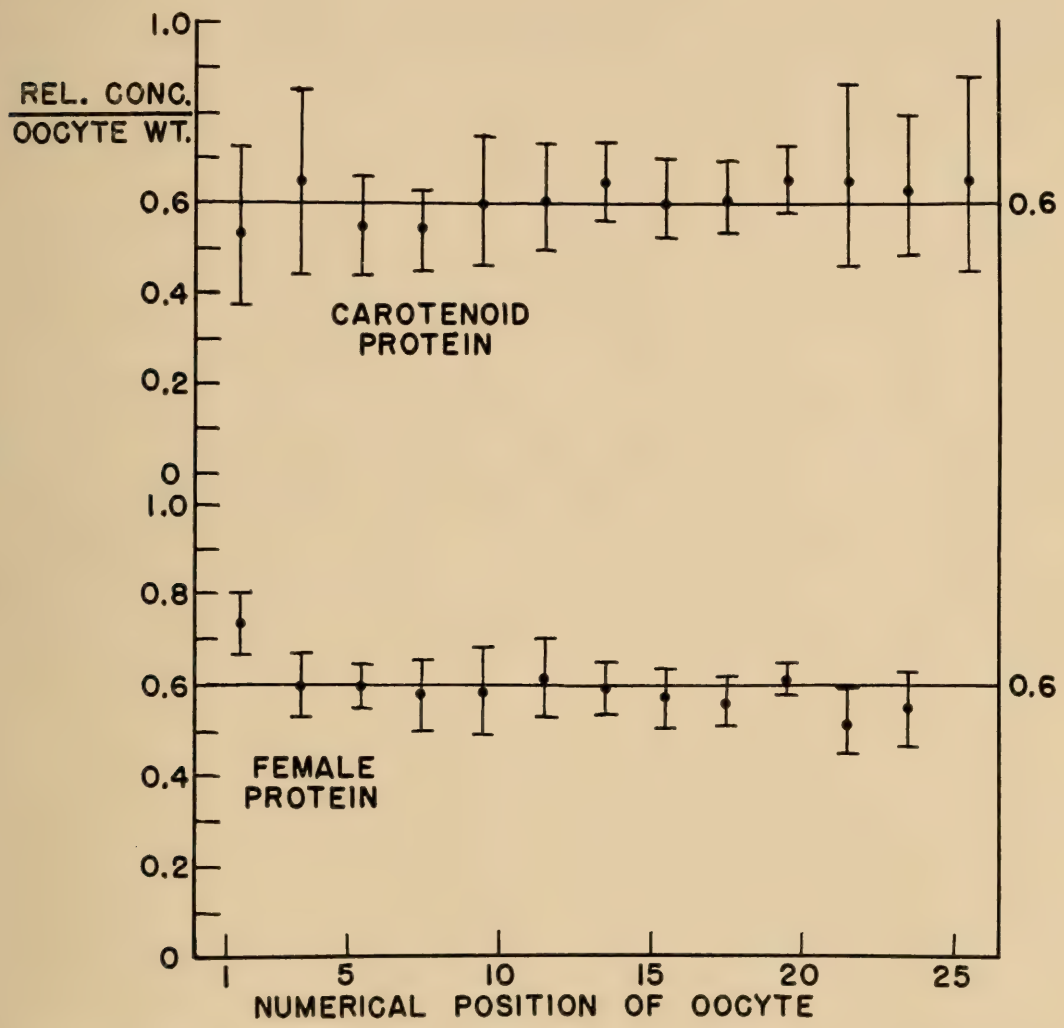


FIGURE 8. Relative concentrations of the carotenoid and female proteins in successive pairs of oocytes dissected from mature moths. The points represent the mean values for five different animals. The bars indicate 95% confidence intervals. The relative concentration of the protein in an extract of standard volume was divided by the fresh weight in milligrams of the oocytes before extraction. Reference lines are arbitrarily placed at 0.6.

procedure which has been found to lyse the yolk spheres and thus to release their stored proteins. The concentrations of female and carotenoid proteins in the extracts were determined with antiserum-agar tests.

From the results summarized in Figure 8, it is apparent that the amount of female protein extracted per mg. of mature oocyte tended to remain constant along the length of the ovariole. While there may have been some decline, this was of questionable significance and it did not approximate the 80% decrease in the

concentration of female protein which occurs in the blood. A similar relationship was found for the carotenoid protein. Despite the approximate 20% decrease in the level of carotenoid protein in the blood, the concentration of this protein in the oocyte tended to remain constant, and perhaps even rose slightly, along the length of the ovariole (Fig. 8).

When considered independently, these results suggest that the rates of accumulation of the two proteins are not affected by the decrease in their concentrations in the blood. However, a comparison of growth rates indicates that the time available for protein accumulation is longer in the later oocytes than in the first oocyte. For instance, the twentieth oocyte required 12 days to increase in volume from 0.01 $mm.^3$, the volume of the smallest oocytes observed to contain yolk, to 3.35 $mm.^3$, its volume at maturity. Due to its higher growth rate the first oocyte requires only 9 days for the same increase in volume. Thus, while the concentration attained by the proteins may be the same in the two oocytes, the time required to achieve this concentration is 33% longer in the twentieth oocyte.

It therefore seems likely that naturally occurring reductions in the concentrations of the carotenoid and female proteins in the blood result in their being accumulated by the oocyte at slower rates. This, in turn, could account for the slower growth rate and smaller size of the oocytes which produce yolk at the end of metamorphosis. However, alternative explanations are possible; for instance, the results summarized in Figure 8 would also be expected if the rate of protein accumulation were governed primarily by the rate at which space is provided for the protein in the oocyte. In such a case, the rate of protein accumulation would be determined by the growth rate of the oocyte, which is the inverse of the relationship proposed above. It therefore proved desirable to seek an independent test of the extent to which deficiencies of a blood protein can impair the growth of the oocyte.

6. *Oocytes produced by ovaries transplanted to males*

Experimental evidence that the level of female protein in the blood may affect the growth of the oocyte was derived from ovarian transplantation experiments. Kopeć (1911) observed in a number of Lepidoptera that the oocytes produced by ovaries which had been transplanted to males are considerably smaller than those produced by ovaries in females. A similar effect has been observed in Cecropia (Telfer, 1954) and is demonstrated here for the Polyphemus moth. The following experiments indicate that this effect is due to the male's deficiency in a factor which is present in the blood of female pupae.

Single ovaries dissected from diapausing female pupae of the Polyphemus moth were transplanted to the haemocoeles of diapausing male pupae. Half of the male pupae were then injected with the blood of male pupae and the other half with the blood of female pupae. The volume of the blood injected was such that the recipient's blood contained a $\frac{1}{5}$ to $\frac{1}{10}$ dilution of the injected blood. Fifteen of the males survived the surgical procedures and completed their development into moths. In 13 of these the implanted ovary, which was dissected from the moth, contained oocytes that had completed their development, as was indicated by the presence of a chorion.

The presence of blood from female pupae enhanced the size attained by the oocytes developing in males. In the males transfused with male blood the average

weight of the mature oocyte was 2.5 mg., the values ranging from 1.9 to 2.8 mg. In those transfused with female blood, the average weight was 3.6 mg. with a range of 2.2 to 4.8 mg. The variance in the latter case was significantly greater ($p = 0.04$) than that for the male-transfused animals—a fact which suggests that there were inconsistencies in the effective level of female blood received by the host. In any case, 6 of the 8 males transfused with female blood produced oocytes which weighed 3.8 mg. or greater and which were thus within the size range of oocytes produced by normal ovaries in Polyphemus females.

Blood taken from female pupae—a stage, incidentally, during which yolk formation does not occur—thus contains something which is lacking in the male and which enhances the formation of yolk. That the female protein is the responsible agent is suggested by the fact that it is approximately one thousand times more concentrated in the blood of female pupae than of males (Telfer, 1954), and that it is accumulated by the growing oocytes in substantial quantities.

Discussion

1. *Comparison with yolk formation in other animals*

The growth rate of an oocyte might be expected to serve as a valid index to the rate of yolk formation only in oocytes whose cytoplasm becomes nearly close-packed with yolk bodies—as in the case of moths and birds. Even in moderately yolky oocytes, however, yolk formation, which is limited to the final stages in the total differentiation of the oocyte (Wilson, 1925), may be the primary cause of the volume increase occurring at the time.

Despite the hazards in this interpretation, comparisons of the Cecropia oocyte with several other oocytes whose growth has been described indicate clearly that the rate of yolk formation varies widely from species to species. Although, to our knowledge, the growth of the amphibian oocyte has not been the subject of an analysis in which the parameter of time was measured, one can presume that its growth during yolk formation is relatively slow from the fact that it spends several months in the process (*cf.*, Grant, 1953). By contrast the process is rapid in the chicken and in the fruitfly. In chickens the absolute growth rate of the oocyte during yolk formation reaches an astonishing 5 grams per day, while the maximum percentage increment in volume has been measured at 284% per day (Romanoff and Romanoff, 1949). The relatively minute oocyte of *Drosophila melanogaster* completes yolk formation in an estimated 4.7 hours and during this time its volume doubles approximately once every 0.7 hours (King, 1957). The Cecropia oocyte with its daily increment in volume of 50–100% per day appears to be intermediate between these extremes.

Variations such as these are presumably adaptations to various factors in the over-all economy of the organism. Whatever their explanation, it is apparent that yolk formation can be a rapid process, resulting in both absolute and relative growth rates which may be among the fastest for metazoan cells.

2. *Conclusions concerning the mechanism of yolk formation in insects*

Data concerning the growth of the oocyte in *Drosophila melanogaster* (King, 1957) are sufficiently extensive to allow a detailed comparison with that of Cecropia. The growth curves of these two oocytes during their period of yolk formation have

two characteristics in common, one of which is the abruptness with which growth terminates. There is no period of senility detectable in the yolk-producing mechanism of insects. The correlation between the termination of growth and the first appearance of the chorion in Cecropia suggests that the two events may be causally related to each other.

A second similarity is the apparent exponential character of oocyte growth during yolk formation. A constant relative growth rate, which is implied by this result, is frequently found in biological systems and can in many cases be interpreted as indicating that the products of growth in turn reproduce. Such an interpretation is not plausible in the case of yolk formation; it would require that yolk be produced throughout the substance of the existing yolk mass, whereas evidence from a number of organisms with relatively yolky oocytes indicates that new materials are deposited primarily at the periphery of the cell.

Lipophilic dyes injected into the chicken (literature reviewed by Romanoff and Romanoff, 1949) and radioactive amino acids injected into frogs (Kemp, 1955) both localized at the periphery of the oocyte rather than throughout its substance. Evidence that the yolk spheres of the insect oocyte grow primarily at the periphery of the cytoplasm is suggested by the cytochemical localization of a number of constituents of the yolk (Bonhag, 1955, 1956; Telfer, 1958). These observations make it likely that the exponential increase in the yolk mass of the insect oocyte results, not from self-reproduction of the mature yolk bodies but from an exponential increase in a peripherally-located mechanism for the formation of yolk. Whether this mechanism is, simultaneously, a self-reproducing mechanism remains, however, a matter of conjecture.

If equation (1) is in fact an accurate description of the growth of the insect oocyte, an additional characteristic pertinent to investigations of the mechanism of yolk formation can be proposed. The volume of the Cecropia oocyte is approximately 400 times greater at the conclusion of yolk formation than at its inception. If follows that the absolute rate of yolk formation also increases by a factor of 400. If one assumes that the oocyte has an approximately constant shape during yolk formation, its surface area increases by a factor of $400^{2/3}$, or only 50. Thus a unit of surface area, or perhaps of the cytoplasm underlying the surface, must produce yolk 8 times faster just before the conclusion of yolk formation than just after its inception. Either the density or the rate of activity of the yolk-producing elements at the surface of the oocyte must also increase by a factor of 8, a fact which could prove useful in the cytological identification of the yolk-producing mechanism. Finally, a similar increase must occur in the rate of penetration per unit of oocyte surface area by blood proteins and by all other raw materials required in the formation of yolk.

3. *The role of blood protein accumulation in yolk formation*

As will be demonstrated more fully in a later paper, the blood proteins accumulated by the Cecropia oocyte are confined to the protein yolk spheres which comprise the bulk of the mass of the oocyte. The process of yolk formation in this insect thus entails the transfer of proteins across the cell surface and their incorporation into a cytoplasmic particle. It is understandable, therefore, that the growth of the yolk mass might be sensitive to the availability of blood protein.

Of two normally occurring blood proteins and five artificially introduced proteins thus far studied (Telfer, 1960), the female protein is the most avidly accumulated, attaining a concentration ratio (oocyte : blood) of 20 : 1. The evidence presented here suggests that deficiencies in this protein can impair the growth of the oocyte. Oocytes reaching maturity in the low female protein environment which prevails at the termination of metamorphosis grow at a slower rate and are smaller at maturity than oocytes which grow during an earlier and more bountiful period. In addition, oocytes developing in males which normally contain only traces of female protein in their blood attain a 40–50% larger size if the host has been injected with the blood of female pupae. There is thus a correlation between the availability of female protein in the blood and the size attained by the oocyte. Whether the correlation results from the influence of the female protein alone on yolk formation is a question whose answer must await the isolation of this protein in experimentally useful amounts. It is probable however that we are dealing here with the effects of a blood protein on the growth of the oocyte and the formation of yolk.

Summary

1. The earliest oocyte to differentiate in an ovariole of the Cecropia moth grows during the final week of its development with a daily increment in volume which was calculated to be 91% per day. Growth during this period is due primarily to the accretion of yolk spheres. It terminates abruptly when the chorion appears at the surface of the oocyte.

2. Successive oocytes in an ovariole lag behind each other in their development by an average of approximately 5 hours; the first oocyte terminates growth more than 6 days earlier than the thirtieth oocyte. As a consequence, the first oocyte produces yolk at a time when at least two prospective yolk proteins are present in the blood at higher levels than those prevailing during the growth of the later oocyte.

3. Correlated with a decline in the level of the two blood proteins are reductions in the growth rates of successive oocytes in the ovariole and in the final size which they attain. Experimental results suggest that depletion of the female protein in the blood may be one of the primary causes of the reduced vigor with which the later oocytes grow.

4. The final concentrations of the two proteins were the same in successive oocytes in the ovariole. Thus, while the normally occurring depletion in the blood's supply of prospective yolk proteins may lead to a reduction in the rate and amount of yolk formation in an oocyte, it does not affect grossly the quality of the yolk produced.

5. From a consideration of the oocyte growth curve and of cytological observations reported for other yolk oocytes, it is proposed that yolk is formed in the Cecropia oocyte by an exponentially increasing mechanism which is probably located at the periphery of the cell.

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